12. S. Simler, L. Giesieski, M. Maitre, et al., Biochem. Pharmacol., 22, 1701 (1973).
13. V. V. Zakusov, S. N. Kozhechkin, T. A. Voronina, et al., Arch. Int. Pharmacodyn., 229, 313 (1977).
14. V. V. Zakusov, Pharmacology of Synaptic Transmission, Pergamon Press, New York (1980).

EFFECT OF ENKEPHALINS ON CENTRAL REGULATION OF THE HEMODYNAMICS

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Recent investigations have revealed a high density of opiate receptors and a high concentration of enkephalins in brain structures concerned with the central regulation of the hemodynamics [6, 16]. The role of endogenous opioid regulators in the control of the cardiovascular system has virtually not been investigated. Isolated observations [7, 13] have been made on anesthetized animals, although anesthetics are known to modify the processes of central regulation of the hemodynamics considerably [3], and part of the anesthetic effect is mediated through an opiate mechanism and is abolished by naloxone [4].

The aim of the present investigation was to analyze the hemodynamic effects of leu- and met-enkephalins in unanesthetized animals. The structure of baroreceptor reflexes and responses of the cardiovascular system to stimulation of hypothalamic emotiogenic zones was assessed.

EXPERIMENTAL METHOD

Experiments were carried out on eight unanesthetized cats under free behavior conditions. Aortic and venous catheters were introduced into the animals 3-5 days before the beginning of the experiments, and a cannula was introduced into the fourth ventricle with coordinates P = 11, L = 0, H = −5. Monopolar nichrome electrodes 150 μ in diameter were introduced with coordinates A = 11-14, L = 1-1.5, and H between −5 and +3. In the course of the experiment the arterial blood pressure (BP) was measured on an EMT-34 electromanometer (from Elema, Sweden), the signal from which was led to an integrating RC circuit with time constant of 2 sec, and from it to an Shch1413 digital voltmeter. Momentary values of the heart rate (HR) were determined by a digital pulsotachometer, triggered by the pulse wave of BP. To record motor activity the animal was placed on the platform of a strain gauge with four semiconductor Yu-12A tensometric resistors. The baroreceptor reflex was tested by intravenous injection of phenylephrine at the rate of 40 μg/kg/min. The sensitivity of the reflex was determined as the ratio of the increase in the intersystolic interval in milliseconds to the increase in the systolic BP in mm Hg. The parameters of hypothalamic stimulation were 100 Hz, 2 msec, 2-6 V, for 10 sec. All parameters obtained in analog form were recorded on the Mingograph-81 and in digital form on a type 3512 (East Germany) digital printer.

Met- and leu-enkephalins synthesized in the laboratory of peptide synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, were dissolved in sterile physiological saline in a concentration of 100-200 μg (volume of fluid injected 100-200 μl) by means of a microsyringe. Opiate receptors were blocked with naloxone (from Endo Laboratories) injected intracisternally in doses of 50 to 100 μg.

EXPERIMENTAL RESULTS

Injection of met- and leu-enkephalins in a dose of 100 μg into the fourth ventricle caused transient hypertension and tachycardia after 10-15 sec. BP returned to normal after 1-2 min but HR fell (Fig. 1, 2). BP 15-

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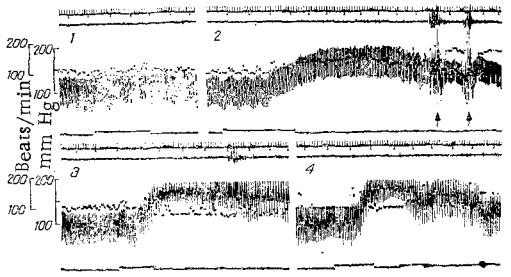

Fig. 1. Effect of intracisternal injection of 100 μg leu-enkephalin on hemodynamic indices and baroreceptor reflex in an unanesthetized cat. 1) Control injection of 100 μl physiological saline; 2) effect of injection of leu-enkephalin, arrows indicate periods of vomiting; 3, 4) effect of injection of phenylephrine, 40 μg/kg, before and 10 min after intracisternal injection of leu-enkephalin respectively. From top to bottom: time marker 1 sec, motor activity, HR, BP, marker of injection of drugs.

20 min after injection of the enkephalines was 102 ± 4.5% and HR 87 ± 3.4% relative to the initial level. Control injection of 100 μl of sterile physiological saline (in both experiments) caused no change in the hemodynamics (Fig. 1, 1). Intracisternal injection of 100 μg naloxone completely abolished the effect of the enkephalin, confirming that the responses of BP and HR observed are connected with activation of brain-stem opiate receptors. Unlike in experiments on anesthetized animals, in which intracisternal injection of opiate peptides or narcotic analgesics gave rise to hypotension [7, 8, 12, 13], in the present experiments BP did not fall after injection of the enkephalins.

Testing the cardiac component of the baroreceptor reflex before and after central administration of the enkephalins showed that they can inhibit the development of baroreflex bradycardia. For instance, whereas the sensitivity of the baroreflex before injection of leu-enkephalin was 6.7 msec/mm Hg, after leu-enkephalin it was only 1.7 msec/mm Hg (Fig. 1). Met-enkephalin was only just over half as effective, for it reduced the sensitivity of the baroreflex only from 5.38 to 2.34 msec/mm Hg. The lower activity of met-enkephalin than of leu-enkephalin when injected intracisternally was noted in [7]. It is an interesting fact that, in relation to their direct vasodilator action (on strips of the pial arteries) met-enkephalin is stronger than leu-enkephalin [10]. The duration of inhibition of the baroreceptor reflex under the influence of enkephalins in the present experiment was 30-40 min, a result which may be linked with the rapid inactivation of the enkephalins. This conclusion is confirmed by data [7] showing the long-lasting change in BP and HR following intracisternal injection of an analog of natural leu-enkephalin with the structure d-ala^2, d-leu^5, which is much more resistant to the action of enkephalin-degrading enzymes [5].

The results of the present experiments with natural ligands of opiate receptors are in good agreement with those obtained by Freye and Arndt [8], who observed depression of the cardiac component of the baroreceptor reflex following perfusion of the fourth ventricle with fentanyl.

It has recently been shown that the mediator secreted by primary baroreceptor afferents is evidently substance P [9]. It can be postulated that inhibition of the baroreceptor reflex may be connected with inhibition of secretion of substance P by primary afferent endings of baroreceptor neurons as a result of their presynaptic inhibition by enkephalin-containing terminals. The basis for such interaction may be the axo-axonal synapses of enkephalin terminals on substance-P-ergic endings discovered in [14, 16]. The possibility that enkephalins may cause presynaptic inhibition has been demonstrated on a nerve–muscle preparation of the rat vas deferens [15]. The fact that the secretion of substance P is reduced by endorphins in the trigeminal nucleus of rats has been proved experimentally [11].

Considering the important role of the hypothalamus in the integration of somatoautonomic responses, the influence of met- and leu-enkephalins on hemodynamic reflexes to stimulation of the emotiogenic zones of the hypothalamus was studied. Leu-enkephalin increased the amplitude of the pressor reflexes on average to 169 ± 22% of the initial level. The greatest increase was observed in reflexes combined with responses of "rage" type. Met-enkephalin did not increase the amplitude of the pressor reflex (Fig. 2). The facilitatory

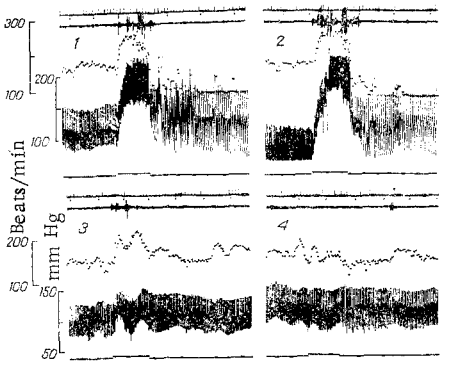

Fig. 2. Changes in pressor reflexes evoked in the same animal by stimulation of the same hypothalamic "point" under the influence of enkephalins. 1) Before, 2) after intracisternal injection of 100 μg leu-enkephalin. Parameters of stimulation: 100 Hz, 2 msec, 6 V; 3) before, 4) after intracisternal injection of 100 μg met-enkephalin. Parameters of stimulation 100 Hz, 2 msec, 4 V. Experiment with met-enkephalin carried out 7 days after experiment with leu-enkephalin. From top to bottom: time marker 1 sec, motor activity, HR, BP, marker of stimulation.

effect of leu-enkephalin on vascular reflexes to stimulation of the hypothalamus was discovered by the writers for the first time. Data on the effects of activators of brain-stem opiate receptors on vascular reflexes reported previously do not contradict the results of the present experiments. For instance, in experiments on unanesthetized decerebrate cats morphine and trimeperidine increased by 50-150% the amplitude of pressor reflexes to stimulation of different zones of the medulla [2]. The effect of the narcotic analgesics was exerted at the level of the medulla, for it was not manifested on pressor reflexes in spinal animals.

Activation of opiate receptors at the level of the medulla in unanesthetized cats is thus accompanied by inhibition of the cardiac component of the baroreceptor reflex and by an increase in the amplitude of vascular responses evoked by hypothalamic stimulation. This type of structure of autonomic reflexes is very characteristic for responses of "rage" type, in which pain sensitivity is depressed. The results confirm the hypothesis put forward previously that endogenous opiate peptides may participate in the integration of autonomic responses in affective states [1].

LITERATURE CITED

1. A. V. Val'dman and Yu. D. Ignatov, Central Mechanisms of Pain [in Russian], Leningrad (1976).
2. G. V. Kovalev, in: Research in the Pharmacology of the Reticular Formation and Synaptic Transmission [in Russian], Leningrad (1961), pp. 149-163.
3. V. M. Khayutin, Vasomotor Reflexes [in Russian], Moscow (1964).
4. V. A. Berkowitz, S. H. Ngai, and A. D. Finck, Science, 194, 967 (1976).
5. D. H. Coy, A. J. Kastin, and A. V. Schally, Biochem. Biophys. Res. Commun., 73, 632 (1976).
6. R. Elde, T. Hökfelt, O. Johansson, et al., Neuroscience, 1, 349 (1976).
7. W. Feldberg and E. Wei, J. Physiol. (London), 280, 18P (1978).
8. F. Freye and J. O. Arndt, Arch. Pharmak. exp. Path., 307, 123 (1979).
9. R. A. Gillis, C. J. Helke, B. L. Hamilton, et al., Brain Res., 181, 476 (1980).
10. J. H. Hanko and J. E. Hardebo, Eur. J. Pharmacol., 51, 295 (1978).
11. T. M. Jessell and L. L. Iversen, Nature, 268, 549 (1977).
12. M. Laubie, H. Schmitt, J. Canellas, et al., Eur. J. Pharmacol., 28, 66 (1974).
13. M. Laubie, H. Schmitt, M. Vincent, et al., Eur. J. Pharmacol., 46, 67 (1977).
14. A. Ljungdahl, T. Hokfelt, and G. Nilsson, Neuroscience, 3, 861 (1978).
15. A. MacDonald, J. C. McGrath, and R. A. Murdoch, Br. J. Pharmacol., 68, 179 (1980).
16. V. M. Pickel, J. H. Tong, S. E. Leeman, et al., Brain Res., 160, 387 (1979).